의학박사 학위논문

Sirolimus and Metformin Synergistically Inhibits Colon Cancer *In vitro and In vivo*

생체 외 및 생체 내 실험을 통한 시롤리무스와 메트포민의 대장암 억제 시너지 효과

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Nadiar Mussin

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Sirolimus and Metformin Synergistically Inhibits Colon Cancer *In vitro and In vivo*

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The Department of Surgery
Seoul National University
College of Medicine

ABSTRACT

We estimated the effect of various immunosuppressants (IS) and metformin to provide theoretical background of optimal therapeutic strategy for de novo colon cancer after liver transplantation (LT). Three colon cancer cell lines (HT29, SW620 and HCT116) were used in in vitro studies. HT29 was also used in BALB/c-nude mice animal models. Following groups were used in both in vitro and in vivo studies: sirolimus (S), tacrolimus (T), cyclosporin A (CsA), metformin (M), metformin/sirolimus (Met/S), metformin/tacrolimus (Met/T), and metformin/cyclosporin A (Met/CsA). 3-(4 5-Dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide (MTT) assay was performed and Western blot analyses were performed for mTOR pathway proteins, apoptosis proteins, and EMT proteins. Tumor volume was measured for 4 weeks after inoculation. MTT-assay revealed significant cell viability inhibition in all three colon cancer cell lines in groups of S, M, and Met/S. Of note, group Met/S showed synergistic effect compare to M or S group. Western blot analysis showed significant low levels of all investigated proteins in groups of S and Met/S in both in vitro and in vivo experiment. Tumor growth was significantly inhibited only in the Met/S group**.** Combination of Met and S showed the most potent inhibition in all colon cancer cell lines. This finding might have

application for de novo colon cancer.

Keywords: Liver transplantation, immunosuppression, de novo colon cancer, metformin

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Chapter 1

Chapter 2

LIST OF ABBREVIATIONS

Adenosine monophosphate-activated protein kinase (AMPK)

Calcineurine inhibitors (CNI)

Liver transplantation (LT).

Mammalian tagrget of rapamycin (mTOR)

Post-transplant lymphoproliferative disorders (PTLD)

Polycystic ovary syndrome (PCOS)

INTRODUCTION

Tremendous progresses have been made in surgical technique, perioperative treatment, and immunosuppressive therapy in liver transplantation (LT). The incidence of early complications such as infection, bleeding, rejection, wound healing, and others have decreased recently. However, late complication such as post-transplant de novo cancers has become one of the leading causes of late mortality after liver transplantation (1, 2).

LT is associated with a 2- to 7- fold higher risk of de novo malignancy (3) after adjusting for age and gender. The incidence of LT has been reported to be ranging from 4% to 16% depending on various factors such as the length of follow-up, age distribution of recipients, types of immunosuppressive regimens, and geographic location (4, 5). In Western countries, the leading type of de novo malignancy has been reported to be post-transplant lymphoproliferative disorders (PTLD) and skin cancer (6). However, solid organ cancer is dominant in Asian countries (7). In Korea, de novo solid malignancy after LT including colorectal malignancy is common and its prevalence is gradually increasing (8, 9). Furthermore, de novo malignancy shows more aggressive nature and growth compare to malignancy in non-transplant

setting patients (10).

Park H.W. in single-center study showed that in period between January 1998 and December 2008, 1,952 adult orthotopic LT including 1,714 living and 238 deceased donors were performed. Among the 1,952 patients, 44 (2.3%) showed de novo malignancies after a mean posttransplant period of 41 months. Among the 14 types of malignancy the most frequent was stomach cancer (n = 11; 25.0%), colorectal cancer (n = 9; 20.5%), breast cancer (n = 4; 9.1%), and thyroid cancer (n = 3; 6.8%). All these patients subjected to aggressive treatment, including surgery, chemo- and radiotherapy, except for one patient with an aggressive primary liver cancer. Over a mean follow-up of 45 months after diagnosis of de novo malignancy, 13 patients (29.5%) died; the overall 3-year patient survival rate was 67.5%. The relative risk of malignancy following orthotropic LT was 7.7-fold higher in men and 7.3-fold higher in women than the Korean general population (7).Peng J.G. evaluated the incidence of *de novo* malignancy after LT and compare with those among the general Chinese population (11).The incidence rate of *de novo* malignancy was 3.0% (14 in 466 patients). The median elapsed time from transplant to the diagnosis of *de novo* malignancy was 42 months (range, 6 to 106 months). The cumulative risk for development of *de novo* malignancy was 1.6%, 2.7%, and 8.2% at 3, 5 and 10 years after LT, respectively. The patients

were all male. The types of *de novo* tumors included digestive system tumor (8 in 14), lung cancer (2 in 14), urologic neoplasm (2 in 14), and hematologic malignant tumor (2 in 14). Over a mean follow-up of 24 months after diagnosis of *de novo* malignancy, 7 patients (50.0%) died; the overall 5-year patient survival rate was 54.5%. The relative risk of malignancy following LT was 9.5 folds higher than the general Chinese population.

Sapisochin G. reported of evolution and management of de novo neoplasm post-liver transplantation from a single European center (12). The incidence of de novo malignancy post-LT was 9.5%, higher than the tumor incidence described in the general population by age groups.

Haagsma E.B. (13) reported of increased cancer risk after liver transplantation in population-based study. In their study twenty-one of the 174 patients developed 23 malignancies (12%). Skin and lip cancer accounted for 12 of the 23 malignancies (52%). Only one patient had a B-cell lymphoma. The cumulative risk for de novo malignancy was 6, 20, and 55% at 5, 10, and 15 years after transplantation, respectively. The overall relative risk (RR) as compared with the general population was 4.3 (95% confidence interval 2.4±7.1). Significantly increased RRs were observed for non-melanoma skin cancer (RR 70.0), non-skin solid cancer (RR 2.7), renal cell cancer (RR 30.0), and colon cancer (RR 12.5). Multivariate analysis showed that an age.40 years and pre-

transplant use of immunosuppression were significant risk factors.

The leading cause of higher incidence of death in patients with de novo malignancy is its aggressiveness maybe related with immunosuppression. Life-long immunosuppressive therapy is needed even in patients with de novo malignancy (14, 15). Death due to de novo malignancy accounts for more than 20% of deaths during long-term follow up (16). Therefore, optimal immunosuppression is needed to reduce the incidence or increase the survival after the development of de novo malignancy.

Metformin is an oral biguanide agent widely used for treating type 2 diabetes mellitus. Unlike most modern drugs, metformin is therefore derived from a natural product used in herbal medicine and was not designed to target a particular pathway or disease mechanism. It was established as a safe and effective therapy before detailed mechanistic studies became possible and, despite its clinical use for 60 years, its molecular mechanisms of action remain much debated. Metformin works by decreasing intestinal glucose absorption, improving peripheral glucose uptake, lowering fasting plasma insulin levels and increasing insulin sensitivity, which result in a reduction of blood glucose concentrations without causing overt hypoglycemia (17). Metformin has been shown to reduce hepatic glucose production, yet not all of its effects can be explained by this mechanism and there is

increasing evidence of a key role for the gut. At the molecular level the findings vary depending on the doses of metformin used and duration of treatment, with clear differences between acute and chronic administration (18). The mechanisms underlying the anticancer effects of metformin can vary (19). Among these various mechanisms, activation of adenosine monophosphate-activated protein kinase (AMPK) is pivotal (19, 20). In 2001 metformin was reported to activate AMPK in rat hepatocytes and rat liver in vivo (21). Although high concentrations (500 μmol/l) of metformin were required to observe AMPK activation after brief (1 h) treatment of cells, significant effects were observed after incubation for much longer periods with just 20 μmol/l metformin, more compatible with concentrations of the drug found in the portal vein.

It has been known for some time that the intestines may be a target organ for metformin (22, 23), with metformin increasing anaerobic glucose metabolism in enterocytes, resulting in reduced net glucose uptake and increased lactate delivery to the liver. Several recent studies have led to a renewed interest in the gut as a major site of action of metformin and three lines of evidence highlight that the liver may not be as important for metformin action in individuals with type 2 diabetes as commonly assumed. First, the glucose-lowering effect of metformin can only partially be explained by a reduction in EGP,

suggesting other glucose-lowering mechanisms for metformin (24). Second, genetic studies in humans have established that loss-of-function variants in *SLC22A1* (the gene encoding OCT1), which reduce hepatic uptake of metformin (25, 26), do not impact upon the efficacy of metformin to lower HbA_{1c} in individuals with type 2 diabetes (27) . Third, a delayed-release metformin that is largely retained in the gut, with minimal systemic absorption, is as effective at lowering blood glucose as the standard immediate-release formulation in individuals with type 2 diabetes (28).

Since metformin's worldwide spread for over 50 years, numerous studies concerning other potential indications have emerged, which showed that metformin can also be used as an anticancer agent (29), an antiaging agent (30), a cardiovascular protective agent (31), a neuroprotective agent (32) or an optional drug for polycystic ovary syndrome (PCOS) (33).

Antitumor effect of metformin was first discovered on hamsters in 2001. In this experiment, there were two groups of high-fat (HF)-fed hamsters. One group received metformin in drinking water for life (HF + Met group), and the other group served as the control group (HF group). All hamsters were treated with N-nitrosobis-(2-oxopropyl)amine, a pancreatic carcinogen, and after 42 weeks, 50% of the hamsters in the high-fat group developed malignant lesions; however, none was found

in the HF + Met group (P<0.05) (34). A large case-control study in Scotland first showed that metformin reduced the risk of cancer in patients with T2DM (odds ratio [OR] 0.77, 95% CI 0.64–0.92 for any metformin exposure versus no metformin exposure) (35). A representative population prospective cohort study of 800,000 individuals showed that metformin reduced the incidences of several gastroenterological cancers in treated diabetes (total 0.12 (0.08–0.19), colorectal 0.36 (0.13–0.98), liver 0.06 (0.02–0.16), pancreas 0.15 (0.03–0.79)) (36). In addition to the reduction of cancer incidence (37, 38), metformin intake was also associated with a decrease of cancer mortality. Landman et al showed that metformin was associated with lower cancer mortality (hazard ratio [HR] 0.43 [0.23–0.80]) and that the effect was dose dependent (39). A recent meta-analysis concluded that metformin reduced cancer incidence and mortality in patients with diabetes, with overall cancer incidence reduced by 31% and cancer mortality reduced by 34% (29). Furthermore, a meta-analysis (40) suggested that metformin had the greatest benefits as an adjuvant agent in colorectal and prostate cancer treatment, particularly in those receiving radiotherapy. However, the dose of metformin needs to be further explored.

Several studies have indicated that metformin can lower the risk of developing cancers including those of the breast, pancreas, colon, and

the prostate both in vitro and in vivo (41-45). Thus, metformin may have some additional benefits in case of de novo cancer.

However, no optimal immunosuppressant (IS) strategy is available in the setting of de novo colon cancer after LT. The objective of this study was to provide theoretical background of optimal immunosuppressant (IS) strategy for de novo colon cancer after LT.

MATERIALS AND METHODS

Cell lines

Three colon cancer cell lines (HT29, SW620, and HCT116) were used in in vitro studies, all colon cancer cell lines were purchased from KCLB (Korean Cell Line Bank). HT29 colon cancer cell line was also used in BALB/c-nude mice animal models.

Groups according to regimens

In in vitro and in vivo experiments according to immunosuppressant and their combinations with metformin, the following eight groups were used: negative control (C), sirolimus (S) alone, tacrolimus (T) alone, cyclosporin A (CsA) alone, metformin (M) alone, metformin/sirolimus (Met/S), metformin/tacrolimus (Met/T), and metformin/cyclosporin A (Met/CsA).

3-(4 5-Dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide (MTT) assay

Cell viability was determined colonimetrically using MTT-assay after 48 h of incubation. HT29, SW620, and HCT116 cells (3×10^3 cells)

were seeded into 96-well plates and separated into groups based on investigated regimens. The concentrations of immunosuppressants were 5 ng/mL for sirolimus, tacrolimus, and cyclosporin A, and 100 ng/mL for metformin. After different treatments, 20 μl of 5 mg/ml MTT was added to each well (0.1 mg/ml) and incubated for 4 h. The supernatant was aspirated and the formazan crystals in each well was dissolved in 200 μl of dimethyl sulfoxide (Sigma-Aldrich) and incubated at 37°C for 30 min. The absorbance value of each well at wavelength of 570 nm was read on a Microplate Reader**.**

Western blot analysis

Western blot analysis was performed using published procedures (46). Briefly, after cells were incubated with immunosuppressants for 48 h, their total protein was extracted. To isolate protein products from cell cultures and tumor tissues, RIPA buffer (Sigma-Aldrich, St Louis, MO, USA) was used. Whole cell lysates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). After incubation in 5% BSA for 1 hour at room temperature (RT), blots were incubated with monoclonal antibody overnight at 4°C followed by incubation with a secondary antibody (1:2000) for 1h at RT.

Blots were developed using an enhanced chemiluminescence detection kit (ECL, Amersham Pharmacia Biotech, Piscataway, NJ). For protein loading analyses, a monoclonal GAPDH (1:5000) was used. Primary antibodies used in western blot analysis included p-mTOR antibody (1:1000), p-70S6K (1:1000), p-4EBP1 (1:1000), livin (1:1000), survivin (1:1000), E-cadherin (1:1000), transforming growth factor (TGF)-β (1:1000), and pSmad3 (1:1000) antibody (Cell Signaling Technology, Beverly, MA, USA).

In vivo experiment

Animal experimental procedures are approved by SNUH IACUC (IACUC No.15-0301-S1A0). In order to create the mouse model of tumor growth, 48 BALB/c nude mice were inoculated subdermaly with 4×10^6 HT29 cells in both flanks and divided on eight groups by six mice. These mice were raised for 1 week until the tumor became palpable and measurable. After measuring the weight and initial tumor size, these mice were randomly separated into eight groups. They were administrated immunosuppressive and biguanide agents at the following doses: sirolimus (1 mg/kg), tacrolimus (1 mg/kg), cyclosporin A (5 mg/kg), and metformin (250 mg/kg). These mice were fed per os daily for 4 weeks. The weight and tumor size were measured

every 3 days. Approximate tumor size initially and during follow up were calculated using the following formula: Length × Width^2 × 0.5 (Fig. 1A). The tumor volumes of each group were compared at 3 weeks. These animals were sacrificed after 3-4 weeks of treatment. Protein was obtained from tumor tissue and Western blot was performed as described above**.**

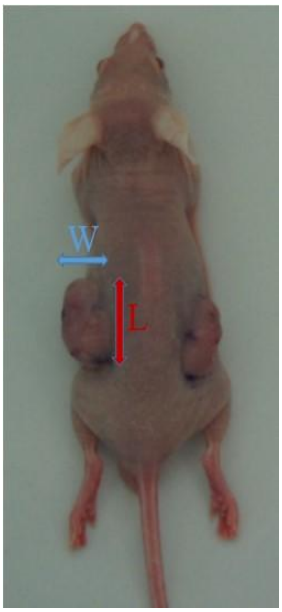


Figure 1 (A)

Statistical analysis

Data are presented as mean ± standard deviation (SD). The mean was compared by Student's t-test. The relative expression of proteins in Western blot was compared by unpaired Student's t-test. The tumor volume at 3 weeks was compared by Mann-Whitney test. Statistically

significance was considered when P value was less than 0.05.

RESULTS

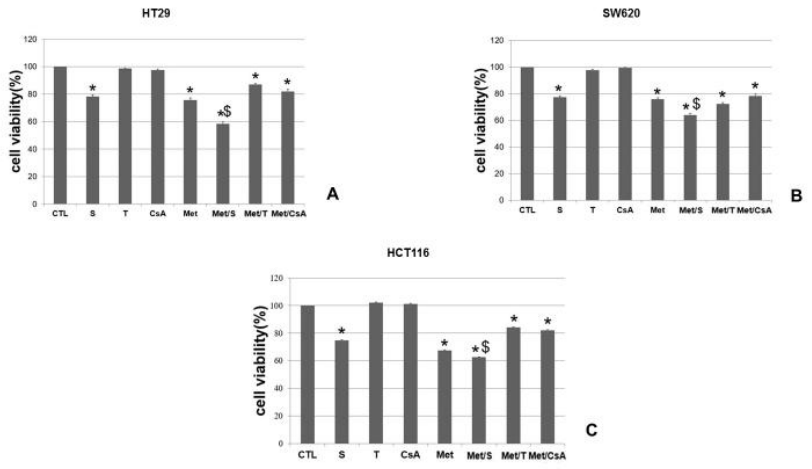
Results

In vitro experiment

1) Metformin and sirolimus significantly inhibited HT29, SW620, and HCT116 cell viabilities

Metformin and sirolimus alone exerted markedly ($P < 0.05$) anti-proliferative effects on HT29, SW620, and HCT116 cell lines in MTT assay. Furthermore, a significant ($P < 0.05$) decrease in the proliferation of colorectal cancer cells was observed in the combined treatment group compared to that of the metformin or sirolimus alone treatment group (Fig. 2A-C). In contrast, treatment with tacrolimus or cyclosporine A alone failed to significantly affect the proliferation of human colorectal cancer cells.

Figure 2



2) Metformin and sirolimus treatment altered the expression of mTOR pathway proteins, EMT related proteins, and apoptosis related proteins in HT29, SW620, and HCT116 cell lines

Western blot analysis showed that treatment with metformin or sirolimus was associated with inhibition of p-mTOR, p-70S6K, and p-4EBP1 proteins. In HT29, SW620, and HCT116 cells, metformin-induced downregulation of p-mTOR was reinforced by co-treatment with sirolimus. However, no significant changes in p-mTOR, p-70S6K, or p-4EBP1 proteins were identified in groups treated with tacrolimus or cyclosporin A alone (Fig. 3 A-C).

Figure 3

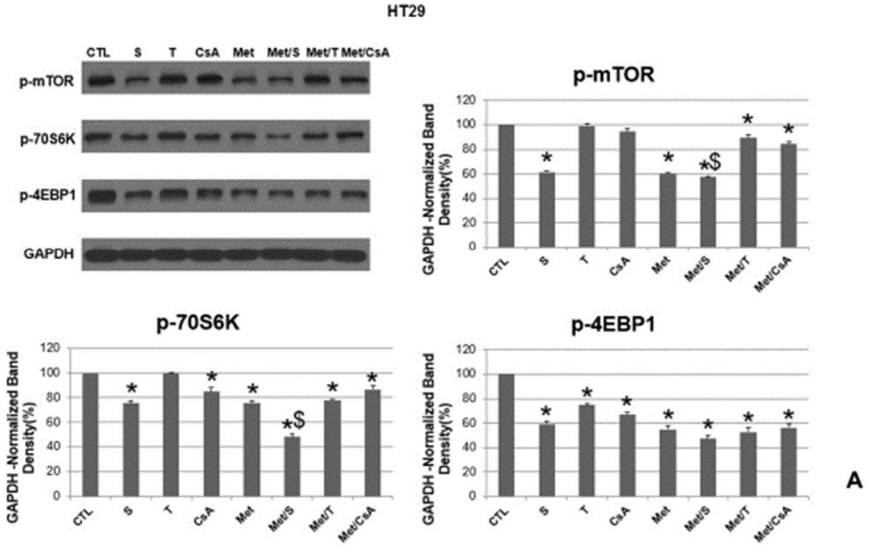


Figure 3

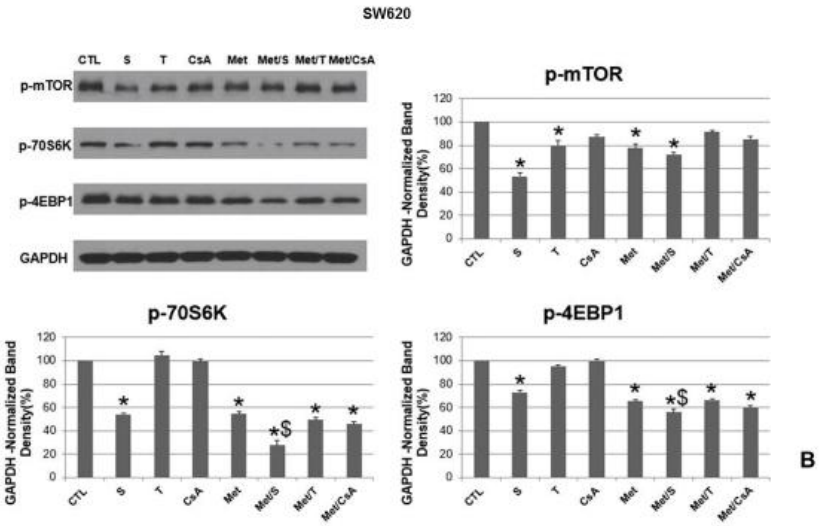
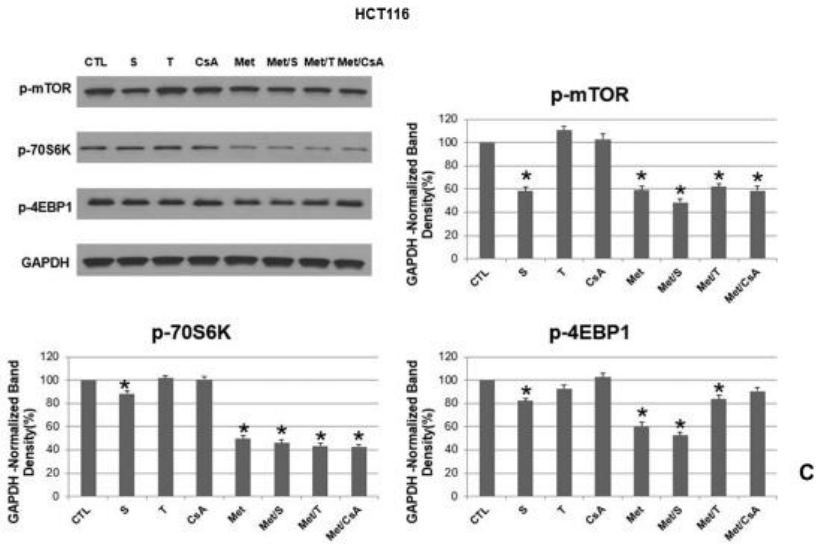


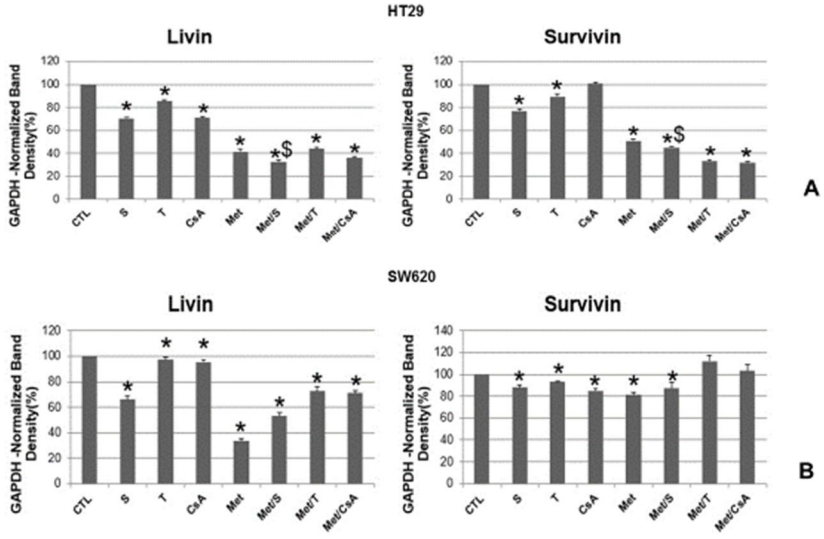
Figure 3



C

In HT29 colon cancer cell line, the combination of metformin and sirolimus showed significant and potent synergistic inhibition effect on apoptosis related proteins. Treatment with sirolimus or metformin alone also showed significant ($P < 0.05$) inhibition on apoptosis related proteins. Livin and Survivin were inhibited by 67.6% and 54.8%, respectively (Fig.4A). However, in SW620 colon cancer cell line, metformin alone showed the most potent and significant inhibition on apoptosis related proteins. Livin and Survivin in SW620 colon cancer cell line were inhibited by 66.8% and 18.8%, respectively (Fig. 4B). In HT116 colon cancer cell line, apoptosis related proteins were also significantly ($P < 0.05$) inhibited by treatment with a combination of metformin and sirolimus. Livin and Survivin were inhibited by 70.4% and 80.4%, respectively.

Figure 4



All three colon cancer cell lines belong to tumors with aggressive growth nature. In that sense, the effect of treatment revealed some interesting findings in EMT-related proteins. All three cell lines well responded to metformin treatment with anti-metastasizing effect. Regarding the expression levels of TGF-β and p-Smad3 in HT29 colon cancer cell line, the combination of metformin and all combinations of immunosuppressants significantly inhibited their expression (Fig. 5A). Of note, the combination of sirolimus and metformin had the best inhibition effect on the expression of TGF-β and p-Smad3 in SW620 colon cancer cell line (Fig. 5B). In HCT116 colon cancer cell line, CNI alone showed worse effect compare to mTOR inhibitor. Metformin alone showed significantly good effect in inhibiting the expression of TGF-β and p-Smad3 (Fig. 5C). Investigation of EMT-related proteins

revealed one interesting fact: in all three cell lines, E-Cadherin was suppressed very slightly, or even overexpressed, especially when cells were treated by CNI alone (Fig. 5A-C).

Figure 5

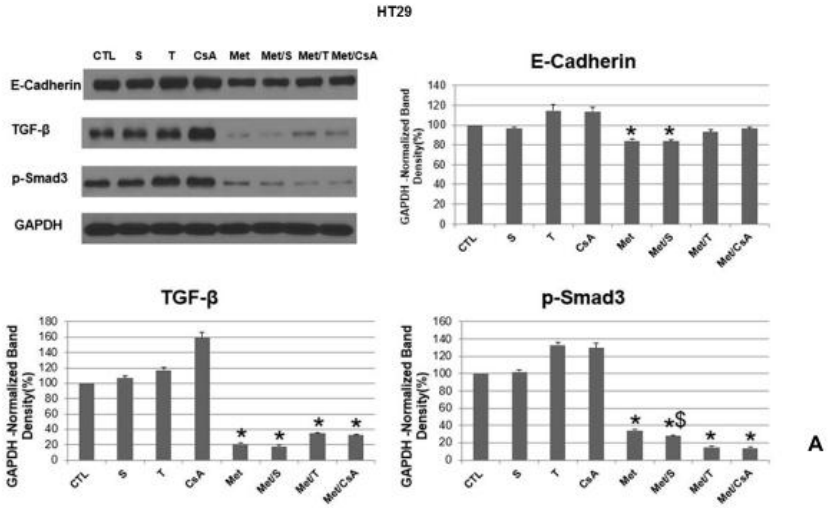
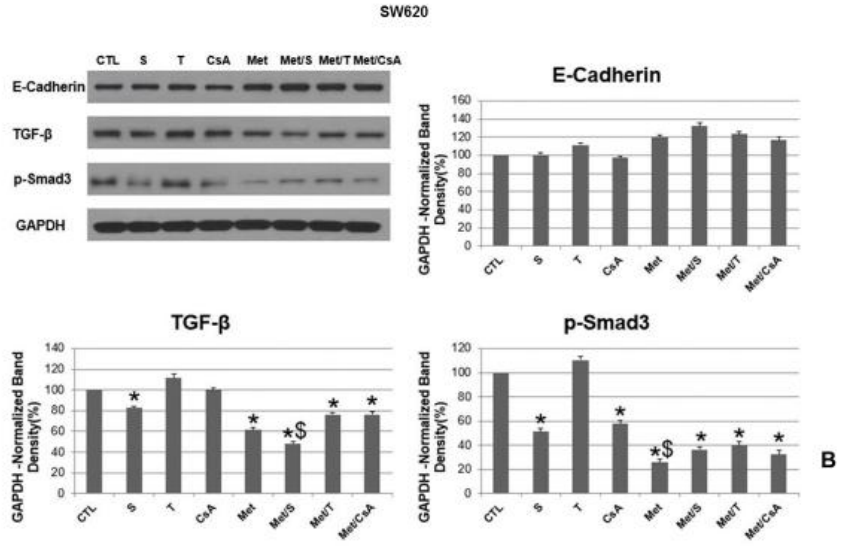


Figure 5



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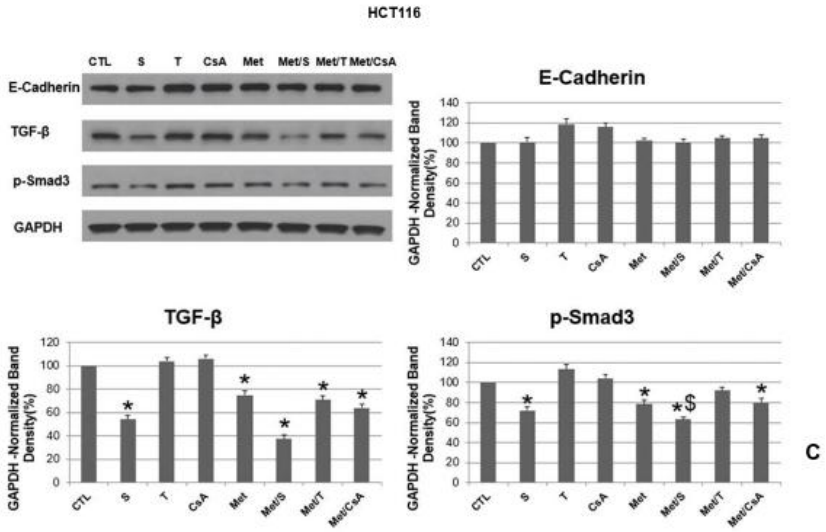


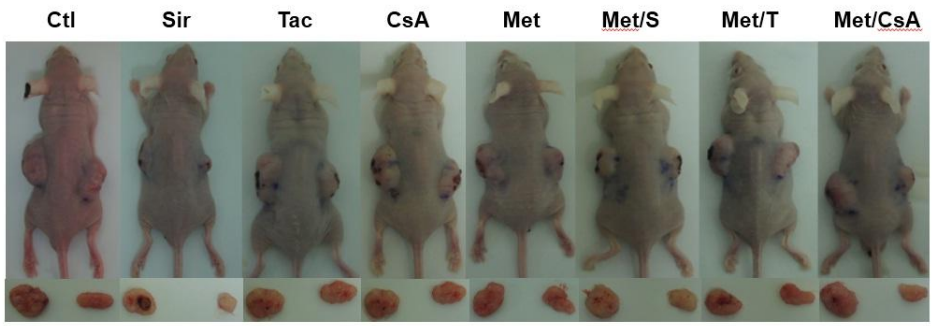
In vivo Experiment

1) Tumor growth was significantly inhibited in Met/S group

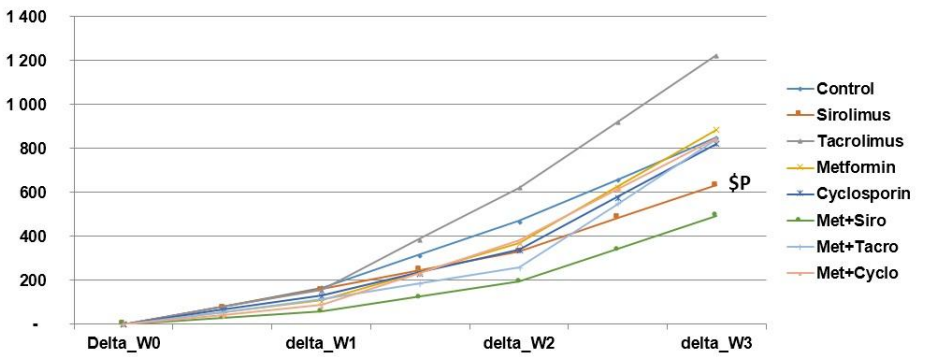
The effectiveness of antitumor therapy was observed in animal models

based on the reduction of tumor size. Our study revealed that tumor growth in group Met/S was significantly inhibited compare to that in control group. However, tumor growth was not significantly inhibited in CNI groups compare to that in the control when CNI was used alone or in combination with metformin (Fig. 1B-C).

Figure 1 B-C



B

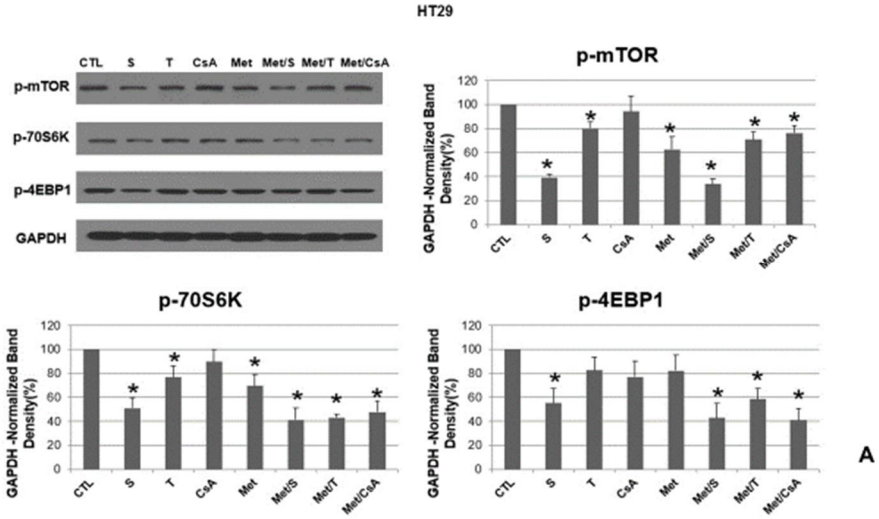


C

2) Expression levels of m-TOR related, apoptosis related, and EMT-related proteins were significantly inhibited in sirolimus and Met/S groups based on Western blot using tissue samples

As mentioned above, the HT29 colon cancer cell line was used in animal models. After 4 weeks of treatment, all experimental models were sacrificed and tumors were dissected and the expression levels of m-TOR related, apoptosis related, and EMT related proteins were determined by western blot analyses. Although the expressional level of m-TOR related, apoptosis related, and EMT related proteins were affected by treatment with cyclosporine A, the differences were not statistically significant (Figure 6A-C). However, in all tissue samples, the expressional level of m-TOR related, apoptosis related, and EMT related proteins were significantly ($p < 0.05$) inhibited by treatment with sirolimus and Met/S. The inhibition effect of metformin alone was less than that of sirolimus alone.

Figure 6



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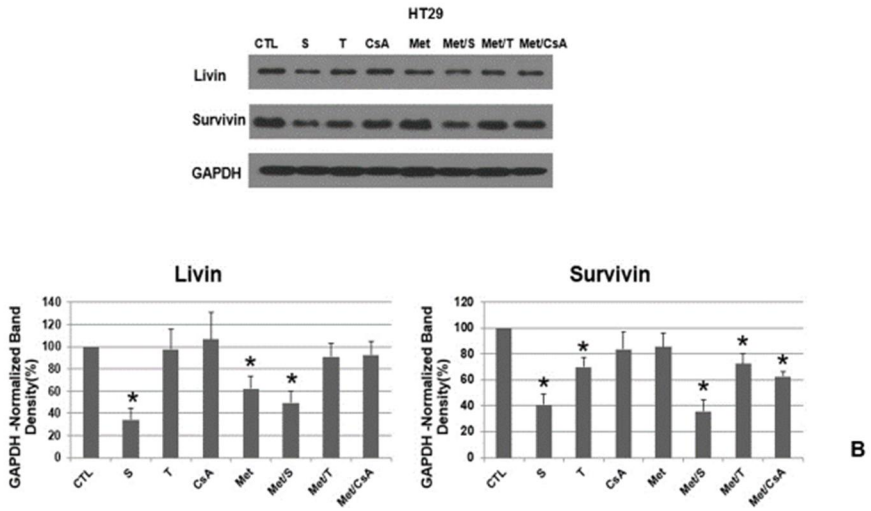
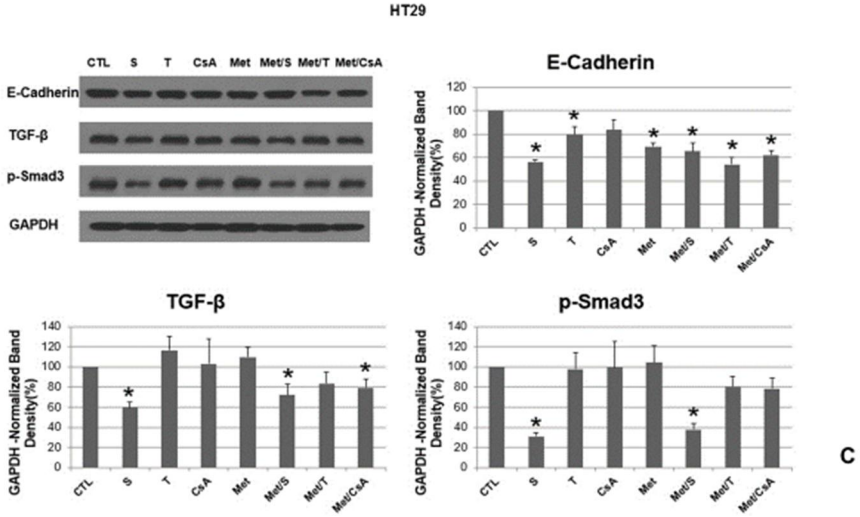


Figure 6


HT29
CTL
S
T
CsA
Met
Met/S
Met/T
Met/CsA
E-Cadherin
TGF-β
p-Smad3
GAPDH
E-Cadherin
TGF-β
p-Smad3
GAPDH -Normalized Band Density(%)

C

DISCUSSION

Immunosuppression therapy used in transplantation is associated with increased incidence of various cancers. Remarkably, it has been reported that patients who have undergone LT for primary sclerosing cholangitis appear to be at an additional increased risk for developing de novo malignancy as high as 9.6% compared to LT patients for other causes (47). Skin cancers and lymphoproliferative diseases are the most common malignancies in Western countries (4) while solid cancers including colon cancer are more common in Asia (7). De novo colon cancer is mostly diagnosed between 16 and 50 months after LT. It is associated with a worse prognosis compared to the general population (48). Therefore, the role of de novo colon cancer after LT as a health concern in liver transplant recipients is steadily increasing.

Nowadays, all LT centers have their own immunosuppression strategies. Generally, they are quite similar in the usage of CNI. There are a few guidelines for cancer surveillance or optimal IS-regimens for de novo colon cancer (49). Especially, no studies have compared various IS on de novo cancers including colon cancer which is reported in up to 0.6% in the LT population (50). Our study is the first to evaluate anti-tumor effect of various IS with or without metformin on three different colon cancer cell lines.

We found that a combination of sirolimus and metformin showed the best anti-tumor effect on colon cancer cell lines. In in vitro experiment, metformin and sirolimus showed significant and synergistic effect in suppressing cell viability and inhibiting the expression levels of m-TOR pathway related, apoptosis related, and EMT related proteins in all three colon cancer cell lines (HT29, SW620, and HCT116). The combination of metformin and CNI (tacrolimus, cyclosporin A) failed to show similar synergism. We observed similar results in in vivo experiment. The "per os" treatment with the combination of metformin and sirolimus for 4 weeks dramatically reduced tumor growth likely via inhibiting mTOR pathway proteins and apoptosis related proteins.

CNIs are the most potent and reliable IS in clinical settings. Sirolimus has not been approved by FDA because of higher incidence of acute cellular rejection and other complications associated with its use. Therefore, the most common regimen for long-term survivor is CNI monotherapy (tacrolimus monotherapy). However, CNI is known to increase tumor development and growth (51). Therefore, if de novo cancer is developed in long-term survivals, we need to change the immunosuppressant.

Several experimental and clinical studies have reported the benefits of mTOR inhibitors (sirolimus) compare to CNI in patients after LT in

terms of nephrotoxicity and HCC recurrence (52-54). They can lower the incidence of NODAT and improve insulin requirements in patients with NODAT (55). Therefore, mTOR inhibitors are increasingly used in clinical setting. Several reports have also shown the anti-tumor effect of sirolimus on colon cancer cells (56) (57). However, those reports focused on treatment purpose in non-transplantation setting. Our study is the first one that focuses on the effect of various immunosuppressants on colon cancer cell lines simulating de novo colon cancer after LT.

In this study, we found that sirolimus had significant anti-tumor effect on colon cancer cell lines. Furthermore, our results demonstrated both efficacy and potential benefits of the combination of sirolimus and metformin in inhibiting colon cancer. Thus, a combination of sirolimus and metformin is recommended for patients with de novo colon cancer after LT.

Metformin is the first line of treatment for type 2 diabetes. However, it has been shown significant anti-cancer effect both in vitro and in vivo using various cancer cell lines (58, 59). Metformin is also well-known to possess therapeutic benefits for nondiabetic indications in cardiology (60), gerontology (61), and metabolic syndrome (62) as a diet to lower bodyweight even in euglycemic people without of DM. Therefore, it

can be prescribed together with sirolimus without any further harmful effect.

Through this in vitro and animal study, we provided theoretical background of immunosuppressant regimen for de novo colon cancer. However, further clinical study is needed to prove this result in clinical settings.

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국문 초록

서론: 고형 장기 이식 후 사용하는 면역 억제제는 많은 장기 이식을 가능하게 하였지만 신생 악성 종양 (de novo cancer) 의 발생 율을 높이는 것으로 알려져 있다. 그 중 메트포민의 항암 효과는 현재 보고 되는 상태이나 면역 억제제를 필수적으로 사용하는 이식 환자에서의 효과는 아직 잘 알려져 있지 않다. 이 이론적 배경을 바탕으로 면역 억제제와 메트포민의 암세포에 대한 영향을 알아보기 위해 연구를 시작하였다.

방법: 생체 외 연구를 위하여 세 개의 대장암 세포 주 (HT29, SW620, HCT116) 을 사용하였다. 그 중 HT29 세포 주는 BALB/c-nude 쥐를 이용한 실험 동물 모델에도 이용하였다. 다음과 같은 그룹화를 하여, 생체 외, 생체 내 연구를 시행하였다: 시롤리무스 (S), 타크로리무스 (T), 사이클로스포린 A (CsA), 메트포민 (M), 메트포민/시롤리무스 (Met/S), 메트포민 / 타크로리무스 (Met/T), and 메트포민/사이클로스포린 A (Met/CsA). 3-(4 5-Dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide (MTT) 분석과 웨스턴 블롯 분석을 통하여 mTOR (mammalian Target of Rapamycin) 신호 전달 단계의 단백질 및 세포 자멸사 관련 단백질, 외피-간엽 형질 변환(epithelial-mesenchymal transformation) 관련 단백질의 발현 여부를 조사하였다.

생체 내 효과 분석 시에는, 쥐에 암세포 접종 후, 4 주후에 종양의 부피를 측정하였다.

결과: 세 개의 대장암 세포 주 MTT 분석 결과, S, M, Met/S 그룹에서 의미 있는 세포 성장 저해를 보였으며, Met/S 그룹에서는 M, S 그룹에 비해 시너지효과를 가지며 세포 성장 저해를 보였다. 웨스턴 블롯 분석에서도 S 와 Met/S 그룹에서 의미 있는 관련 단백질 저하를 보였다. 생체 내 분석에서는 오직 Met/S 그룹에서 종양의 크기가 저해됨을 확인 할 수 있었다

결론: 메트포민과 시롤리무스의 조합은 가장 효과적으로 대장암 세포를 억제하는 것으로 보이며, 이는 향후 신생 대장암의 위험을 낮출 것으로 기대한다.

주요어 : 간이식, 면역 억제제, 신생 대장암, 메트포민
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